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Gel chromatography of exo-trimethylenenorbornyl-2-exo phosphates on Sephadex LH-20

Recently gel chromatography has often been employed as a means of investigating the components of a reaction mixture having either high molecular weights or high boiling points. Gel chromatographic separation depends primarily on the difference of molecular weight and is particularly effective for structurally analogous oligomers.

In our laboratory tris-(exo-trimethylenenorbornyl-2-exo)phosphate has been prepared by the condensation of phosphorous oxychloride and 2-exo-hydroxy-exo-trimethylenenorbornane with a base *e.g.* pyridine. However, the triester was obtained in admixture with other types of esters. Therefore, the investigation of the composition of the reaction mixture was necessary to study the reaction conditions and the reaction mechanism.

The present report describes the separation of the above-mentioned reaction mixture by gel chromatography on a lipophilic dextran derivative, Sephadex LH-20, using several solvents.

Experimental

Dimethylformamide, methanol, ethanol, isopropanol, chloroform, dioxane and acetone, all reagent grade were examined for their suitability as developing solvents. A Sephadex column (Model SR 25/100) and a differential refractometer (Waters Associates, Model R-4) were employed as detector. The elution chromatogram was automatically recorded by a recorder (Toa Electronics, Electronic Polyrecorder EPR 2T). Polyethylene tube (1 mm I.D.) was used for connections between each piece of apparatus. Sephadex LH-20 (Pharmacia Fine Chemicals) was equilibrated with the solvent for several hours and then poured into the column. The swollen gel bed was allowed to settle overnight by passing solvent through it. By maintaining a reservoir, which contained the developing solvent, at a set height the flow rate was controlled at 37–40 ml per hour. 70 mg of the reaction mixture from which the reaction solvent was completely removed under reduced pressure were chromatographed as follows: The reaction mixture was dissolved in 0.5 ml of the developing solvent and carefully applied on the top of the column. Each component, as indicated by the recorder, was collected with a measuring cylinder. After removing the solvent, the residue was weighed in order to calculate the recovery and the determination of the components was carried out by means of elementary analysis, mass spectrometry, infrared and nuclear magnetic resonance.

Results and discussion

Three peaks appeared on chromatographing the reaction mixture, using a polar solvent such as dimethylformamide, methanol, ethanol or isopropanol. Fig. 1 shows the chromatogram in the case of dimethylformamide. These three fractions were collected separately and the compositions were determined as shown on Fig. 1. The total recovery was about 97–98%.

In this report the degree of separation between two solutes was expressed in

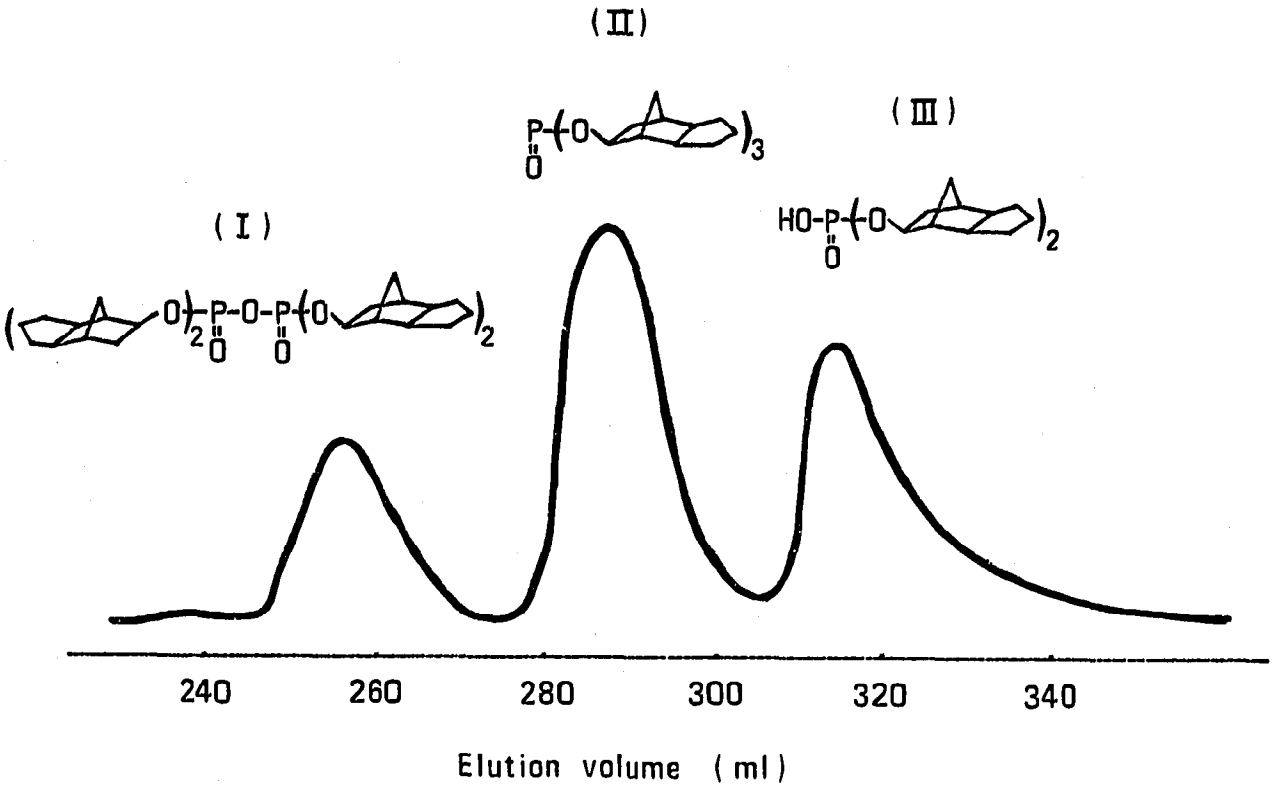

Fig. 1. Separation of the reaction mixture. Chromatographic conditions: Solvent, dimethylformamide; column, 2.5 × 90 cm; flow rate, 38.8 ml/h; sample size, 70 mg. Room temperature.

terms of peak resolution[1]. Table I shows peak resolution between (I) and (II), and (II) and (III) in the solvents examined. The best separation between (I) and (II) was obtained in dimethylformamide which has a high solvent regain value for Sephadex LH-20. With the exception of the alcohols, good peak resolution was also obtained in other solvents. Dimethylformamide also gave the best result for separations between (II) and (III) in a single solvent. In the case of alcohols, the lesser the polarity, the better the peak resolution. When chloroform, dioxane and acetone were used, the peak of (III) did not appear on the chromatogram.

TABLE I

THE INFLUENCE OF SOLVENTS ON PEAK RESOLUTION

Column size: 2.5 × 90 cm; flow rate: 37–40 ml/h; sample size: 70 mg; room temperature.

Solvent	R_{I-II}[a]	R_{II-III}[a]
Dimethylformamide	1.74	1.55
Methanol	0.97	0.31
Ethanol	0.88	0.78
Isopropanol	0.88	1.29
Chloroform	1.26	—
Dioxane	1.36	—
Acetone	1.47	—
Dimethylformamide–dioxane (1:1)	1.71	3.33

[a] Peak resolution values between I and II, II and III.

It has been reported that Sephadex LH-20 adsorbs the molecules containing either a carboxyl or hydroxyl group in some developing solvents such as chloroform and ethanol[2,3]. Therefore, it can be presumed that the results described above were due to an adsorption mechanism similar to those reported.

A better separation between (II) and (III) could be obtained with the mixed solvent, dimethylformamide–dioxane (1:1), rather than with the single solvent, dimethylformamide. Consequently, the mixed solvent was finally used for this gel chromatography. The composition ratio in the reaction mixture could be easily calculated from the individual peak areas in the chromatogram.

Industrial Research Laboratories, HIRONORI TANAKA
Kao Soap Co. Ltd., KAZUO KONISHI
1334 Minato-yakushubata, Wakayama-shi (Japan)

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